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RESEARCH ARTICLE

Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Etamsylate and Tranexamic Acid in Tablet Dosage Form

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ABSTRACT

The present manuscript describe simple, novel, rapid, precise, accurate, specific and cost effective absorbance correction spectrophotometric method for the determination of Etamsylate and Tranexamic Acid in combined tablet dosage form. Here 0.01N Hydrochloric acid used as derivatizing agent and methanol as solvent for making Tranexamic Acid UV detectable. Absorbance correction method involves measurement of absorbance at 305.29 nm for estimation of Etamsylate and measurement of corrected absorbance at 239 nm for estimation of Tranexamic Acid. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines and all validation characteristics were found within the acceptance limits. Thus the proposed method can be successfully applied for simultaneous determination of Tranexamic Acid and Etamsylate in combined tablet dosage form.

KEYWORDS

Etamsylate, Tranexamic acid, Absorbance Correction, Derivatization

INTRODUCTION

Etamsylate (ETS) is chemically N-ethylamine 2, 5-dihydroxybenzenesulphonate.[1] It is a haemostatic agent.[1] Etamsylate reduce bleeding time and blood loss by increasing platelet aggregation mediated by a thromboxane A2 or prostaglandin F2α dependent mechanism and decreased concentrations of 6-oxoprostaglandin F1α.[2] Etamsylate improves capillary wall stability, but does not stabilize fibrin.[3] Etamsylate shown to be effective in reducing blood loss from menorrhagia[4] and after trans-urethral resection of the prostate.[5] Etamsylste is official in British Pharmacopoeia.[6] Individual determination of Etamsylate is carried out by UV[7], HPLC[8] and Electro chemiluminescence.[9]

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A representative structure of Etamsylate was shown in Figure-1.

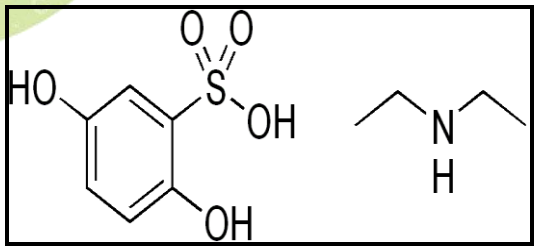

Figure 1: Structure of Etamsylate

Tranexamic Acid (TXA) is chemically trans-4-amino methyl-cyclo hexane carboxylic acid.[1] It is an antifibrinolytic agent.[1] Tranexamic Acid reversibly blocking lysine binding sites on plasminogen and thus preventing fibrin degradation.[10] Tranexamic Acid has been used in heavy bleeding associated with uterine fibroids, neoplasms, gastrointestinal bleeding, hematuria, postoperative bleeding.[11] Tranexamic Acid is

official in British Pharmacopoeia[6] and Japanese Pharmacopoeia.[12] Individual determination of Tranexamic Acid is carried out by LC[12], UV[13], HPLC[14] and LC-MS/MS.[15] A representative structure of Tranexamic acid was shown in Figure-2.

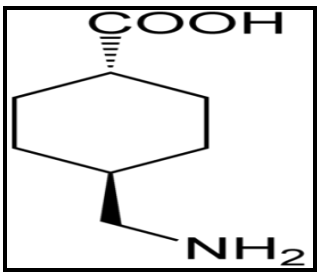

Figure 2: Structure of Tranexamic Acid

ETS and TXA fixed dose combination is used in the management of menorrhagia.[10] The combination therapy is a two pronged approach to control bleeding by the antifibrinolytic action of TXA and achieving hemostasis by improving platelet adhesiveness and restoring capillary resistance by the action of ETS.[10] Literature survey reveals that number of methods such as RP-HPLC[16] and spectrophotometry[17] (simultaneous equation) are reported for simultaneous estimation of ETS and TXA in tablet dosage form. Hence, in the present assay a new simple, accurate, precise and specific UV spectrophotometric (Absorbance Correction) method is developed and validated for simultaneous estimation of TXA and ETS in tablet formulation.

MATERIAL AND METHODS

Instruments

The instruments used for the present study were an UV-visible double beam spectrophotometer (Shimadzu, 1800, Japan) with 1 cm matched pair quartz cell corresponding to 1 cm path length and AUX – 220 single pan electronic digital balance for weighing the materials.

Materials and Reagents

Pure sample of Etamsylate was provided as a gift sample from FDC pharmaceutical limited, Verna industrial estate, Goa, India and Tranexamic Acid was obtained from Jinlan pharma drugs technology co., Limited, Hangzhou, China. A commercial tablet formulation (TRAPIC- E 250mg) was obtained from local market. All the chemicals used were of analytical grade.

Preparation of 0.01N Hydrochloric acid

The solution was prepared by diluting 0.085ml of hydrochloric acid with sufficient distilled water to produce 1000ml.

Preparation of Standard Stock Solution of Etamsylate

Standard stock solution was prepared by accurately weighing 100mg of Etamsylate in 100ml calibrated volumetric flask after that add 5ml of 0.01N hydrochloric acid in it and finally made up the volume with methanol upto 100ml to obtained concentration of 1000µg/ml.

Preparation of Standard Stock Solution of Tranexamic Acid

Standard stock solution was prepared by accurately weighing 100mg of Tranexamic Acid in 100ml calibrated volumetric flask after that add 5ml of 0.01N hydrochloric acid in it and finally made up the volume with methanol upto 100ml to obtained concentration of 1000µg/ml.

Preparation of Working Standard Solution of Etamsylate

Working standard solution was prepared by transferring of 10ml standard stock solution of Etamsylate into 100 ml calibrated volumetric flask and made up the volume with methanol for getting concentration of 100µg/ml.

Preparation of Working Standard Solution of Tranexamic Acid

Working standard solution was prepared by transferring of 10ml standard stock solution of Tranexamic Acid into 100 ml calibrated volumetric flask and made up the volume with methanol for getting concentration of 100µg/ml.

Preparation of Calibration Curve for Etamsylate and Tranexamic Acid

Different aliquots (4.5, 5, 5.5,6, 6.5, 7, 7.5 ml) were taken from the respective working standard solutions (100µg/ml) of both drugs in separate 10 ml volumetric flasks and finally volume was

made up to the mark with methanol to prepare a series of concentrations ranging from 45 to 75µg/ml for both drugs. All dilutions were scanned in wavelength range of 400 nm to 200 nm. The λ-max of Etamsylate and Tranexamic Acid were found to be 305.29 nm and 239 nm respectively. Calibration curve for both drugs were plotted by taking concentration of drug (µg/ml) on X-axis and absorbance on Y-axis. A representative overlain spectrum of ETS (75µg/ml) and TXA (75µg/ml) was shown in Figure-3.

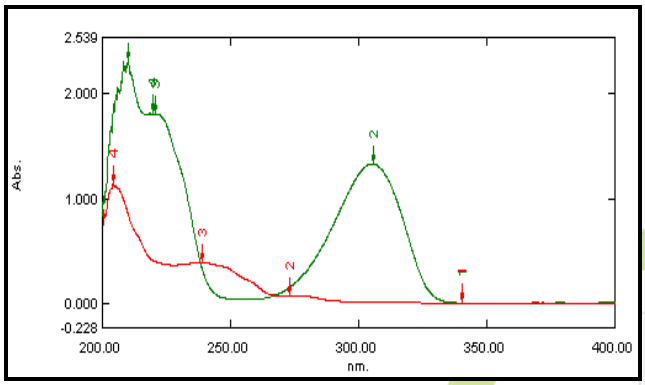

Figure 3: Overlain Spectrum of ETS (75µg/ml) and TXA (75µg/ml)

Estimation of Ethamsyalte and Tranexamic acid in Pharmaceutical Tablets

Twenty tablets were weighed accurately and powdered finely. A quantity of powder equivalent to 50 mg of ETS (50mg of TXA) was transferred into a 100 ml volumetric flask, add 5ml of 0.01N HCl and volume was made up to 100 ml with methanol. The solution was filtered through Whatman filter paper no. 41 to remove particulate matter, if any. Then from above filtered solution 1ml solution was transferred to 10ml volumetric flask and volume was made up to 10 ml with methanol to get final concentration of ETS 50µg/ml and TXA 50µg/ml. The absorbance of sample solution was measured at 305.29 nm and 239 nm against blank. The content of ETS and TXA in tablet was calculated using and absorbance correction method.

Absorbance Correction Method

Absorbance ratio method uses the absorbances at two selected wavelengths, one at λ_{max} of one drug where (239 nm λ_{max} of TXA) other drug also shows considerable absorbance and other being the wavelength at which the first drug has practically nil absorbance (305.29 nm TXA show nil absorbance) is the corrected wavelength. The absorbance of the solution was measured at 305.29 nm and 239 nm and concentration of the two drugs were calculated using following equation:

(A) Concentration of ETS at 305.29 nm

A=abc

Where, A=Absorbance of mixture at 305.29 nm

a= A(1%,1cm) of ETS at 305.29 nm

b= Path length = 1cm

c= Concentration in gm/100ml

Calculate concentration and convert it in µg/ml

(B) Absorbance of ETS at 239 nm

A=abc

Where, A=Absorbance of ETS alone at 239 nm

a= A(1%,1cm) of ETS at 239 nm

b= Path length = 1cm

c= Concentration of ETS at 305.29 nm

(C) Calculation of Concentration of TXA from the Corrected Absorbance at 239nm

Corrected absorbance = A239 (mixture) – A239 (ETS)

Concentration of TA from corrected absorbance,

c = A/ab

Where, A=Corrected absorbance

a= A(1%,1cm) of TXA at 239 nm

b= Path length = 1cm

c= Concentration of TXA in gm/100ml

RESULTS AND DISCUSSION

In proposed method Tranexamic Acid not having any conjugation in it's structure so by addition of 0.01N Hydrochloric acid (5ml) with methanol, conjugation was formed and it was shown absorbance in UV region (200-400). Here esterification of carboxylic acids with alcohols has been observed. Carboxylic acids attacked at

the carbonyl carbon atom of RCOOH with weak nucleophiles, like alcohols (ROH) but the reaction rates were generally too slow to be useful for derivatization of acids. The reaction rate could be increased by acid catalysis, which was usually achieved with mineral acids HCl. Acid catalysis enhanced the carbonyl character of RCOOH by protonation, thus rendering the carbonyl atom more susceptible to nucleophilic attack; it also has the effect of promoting the loss of the leaving group, water, as H_2O is lost more easily than OH-. Generally, derivative agent was added at last stage but in this method it was added in starting stage while stock solution was prepared (1000µg/ml) because catalysis with hydrogen chloride dissolved in the appropriate alcohol gives much better results. The strength and amount of Hydrochloric acid was selected by various trial and error methods.

Method Validation

The developed method was validated according to the International Conference on Harmonization (ICH) guidelines.[18]

Linearity

Linearity was determined over the range of 45 to 75µg/ml for ETS with correlation coefficient 0.9988 at 305.29 nm. Linearity of TXA was determined over the range of 45 to 75µg/ml with correlation coefficient 0.9996 at 239 nm. Regression parameters for ETS and TXA are mentioned in Table-1 and Table-2 respectively. The calibration curve for ETS (305.29 nm) and TXA (239 nm) are shown in Figure-4 and Figure-5 respectively.

Table 1: Linearity data of ETS 305.29 nm

Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Mean (n=3) ±SD	Coefficient of Variance
45	0.795	0.798	0.797	0.797 ±0.001	0.191
50	0.893	0.892	0.898	0.894 ±0.003	0.359
55	0.971	0.974	0.975	0.973 ±0.002	0.214
60	1.059	1.064	1.060	1.061 ±0.002	0.249
65	1.139	1.147	1.146	1.144 ±0.004	0.381
70	1.227	1.224	1.227	1.226 ±0.001	0.141
75	1.293	1.299	1.296	1.296 ±0.003	0.231

Table 2: Linearity data of TXA 239 nm

Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Mean (n=3) ±SD	Coefficient of Variance
45	0.254	0.257	0.265	0.259 ±0.005	2.20
50	0.274	0.283	0.279	0.279 ±0.004	1.62
55	0.299	0.294	0.302	0.298 ±0.004	1.35
60	0.314	0.317	0.319	0.317 ±0.002	0.79
65	0.337	0.329	0.340	0.335 ±0.005	1.70
70	0.359	0.355	0.358	0.357 ±0.002	0.58
75	0.371	0.378	0.380	0.376 ±0.004	1.25

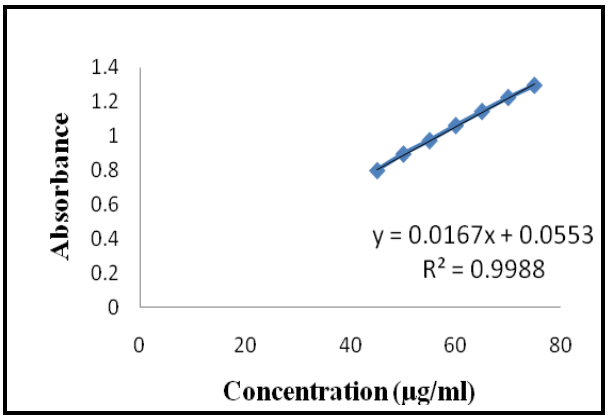

Figure 4: Calibration Curve for ETS (45-75µg/ml)

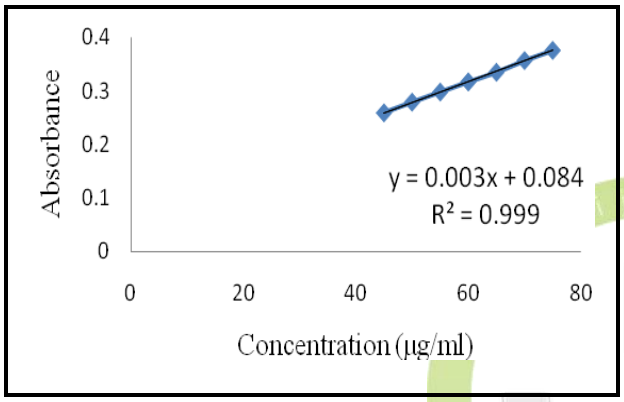

Figure 5: Calibration Curve for TXA (45-75µg/ml)

Precision

Repeatability

Repeatability of the method was determined by analyzing mixed standard solution of ETS and TXA at (60 µg/ml for ETS and 60 µg/ml for TXA) 6 times without changing the parameters of measurement. The results are reported in terms of relative standard deviation (RSD) in Table-3.

Table 3: Precision (Repeatability) data for ETS and TXA

Concentration ETS 60µg/ml and TXA 60µg/ml	Absorbance	
	ETS	TXA
1	1.062	0.319
2	1.056	0.322
3	1.052	0.315
4	1.057	0.313
5	1.050	0.317
6	1.048	0.312
Mean(n=6) ±S.D	1.054 ±0.005	0.316 ±0.003
%RSD	0.489	1.194

Intermediate Precision

The intraday and inter day precision of the proposed method was performed by analyzing the corresponding responses three times on the same day(intraday) and on three different days (interday) over a period of one week for three different concentrations (55,60 and 65 µg/ml) of standard solutions of ETS and TXA .Result was showed in Table-4.

Table 4: Precision (Intraday and Interday) data for ETS and TXA

Concentration taken (µg/ml)		Intraday Precision Mean %Estimated ±%RSD (n=3)		Interday Precision Mean %Estimated ±%RSD (n=3)	
ETS	TXA	ETS	TXA	ETS	TXA
55	55	98.64 ±0.45	98.85 ±0.52	98.80 ±1.82	98.59 ±0.77
60	60	98.71 ±0.38	98.72 ±0.58	99.40 ±0.51	99.33 ±0.97
65	65	98.76 ±0.60	98.60 ±0.38	98.54 ±0.16	99.77 ±1.58

Accuracy

Accuracy was checked by recovery study at 3 different concentration levels, i.e., a multilevel recovery study. The tablet samples were spiked with an extra 80, 100, 120% of standard Etamsylate and Tranexamic Acid, and the mixtures were analyzed by proposed method. Results of the recovery study are shown in table 4 suggested that method was accurate for the simultaneous estimation of ETS and TXA in their combined dosage forms. Result was showed in Table-5.

Table 5: Accuracy (% Recovery Study) data for ETS and TXA

Assay level (%)	Tablet powder taken eq to (mg)		Standard added (mg)		Total drug recovered (mg)		%Recovery of standard added		Mean ± S.D (n=3)	
	ETS	TXA	ETS	TXA	ETS	TXA	ETS	TXA	ETS	TXA
Blank	50	50	0	0	49.20	49.61	-	-	-	-
	50	50	0	0	49.38	49.91	-	-	-	-
	50	50	0	0	49.50	50.29	-	-	-	-
					49.36	**49.94**	-	-	-	-
80	50	50	40	40	89.8	89.6	101.2	99.2	100.6 ± 1.2	99.7 ± 1.49
	50	50	40	40	90.1	89.3	101.8	98.6		
	50	50	40	40	89.1	90.5	99.4	101.0		
100	50	50	50	50	99.0	98.9	99.4	98.1	99.1 ± 0.30	98.9 ± 0.77
	50	50	50	50	98.9	99.7	99.0	99.5		
	50	50	50	50	98.7	99.58	98.8	99.3		
120	50	50	60	60	108.2	109.5	98.0	99.3	99.7 ± 2.0	98.6 ± 0.6
	50	50	60	60	108.8	109.0	99.0	98.5		
	50	50	60	60	110.6	108.7	102.0	98.0		

Table 6: Analysis of Sample by Absorbance Correction method (Assay)

Tablet powder taken eq to (mg)		Amount found (mg/tablet)		% Estimated		Mean (%Estimated) ± S.D (n=3)	
ETS	TXA	ETS	TXA	ETS	TXA	ETS	TXA
50	50	49.08	49.92	98.16	99.84	98.44 ±0.48	99.34 ±0.90
50	50	49.50	49.94	99.00	99.89		
50	50	49.08	49.15	98.16	98.30		

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

$$\mathbf{LOD = 3.3\ X\ \sigma/S}$$

$$\mathbf{LOQ = 10\ X\ \sigma/S}$$

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

LOD and LOQ for Etamsylate were found to be 3.23µg/ml and 9.80µg/ml respectively. LOD and LOQ for Tranexamic Acid were found to be 0.55µg/ml and 1.67µg/ml.

Estimation of Etamsylate and Tranexamic Acid in Tablet Dosage Form

The proposed validated method was successfully applied to the simultaneous determination of Etamsylate and Tranexamic Acid in tablet dosage form. The results of analysis of tablet formulation are shown in Table-6.

Summary

Table 7: Summary of Validation Parameter

Parameter	Absorbance Correction Method	
	ETS	TXA
Linearity Range (µg/ml)	45-75	45-75
Correlation Coefficient	0.9988	0.9996
Accuracy (%Recovery)	99.10-100.60	98.60-99.70
Intraday Precision %RSD	0.38-0.60	0.38-0.58
Interday Precision %RSD	0.16-1.82	0.77-1.58
% Assay	98.44	99.34
LOD (µg/ml)	3.23	0.55
LOD (µg/ml)	9.80	1.67

CONCLUSION

The method was found to be linear between the range of 45-75 µg/ml for TXA and 45-75 µg/ml for ETS. The mean percentage recovery was found 99.10-100.60 for ETS and 98.60-99.70 for TXA at three different levels of standard additions. The precision (intra-day, inter-day) of methods were found within limits (RSD <2%). It could be concluded from the results obtained in the present investigation that the above developed and validated method for the simultaneous estimation of TXA and ETS in tablet dosage form was novel, rapid, simple, accurate and precise and can be used, successfully in other routine laboratory analysis.

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